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Cryptic Trysts, Genomic Mergers, and Plant Speciation

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Summary

It has long been recognized that interspecific hybridization is common in plants, enhancing processes of diversification and speciation. With the widespread utilization of molecular tools, interspecific hybridization – as revealed through incongruence among two or more phylogenetic data sets – is now inferred to be even more prevalent than indicated by morphological and cytogenetic evidence. Using *Gossypium* as an example, we show how multiple molecular markers have implicated a high frequency of historical hybridization between lineages whose modern descendants are strongly isolated by geography and intrinsic genetic barriers. For example, transoceanic dispersal of propagules from Africa to the New World led to the creation of a novel allotetraploid lineage, as well as the introgression of African repetitive elements into a Mexican diploid species. By mechanisms that remain obscure, fully one-quarter of modern *Gossypium* species appear to have experienced historical interspecific cytoplasmic and possibly nuclear introgression. These remarkable observations of interspecific genetic exchange emerge from a genus for which such contact would appear improbable, implying that historical hybridization is a more creative force than suspected in angiosperm evolution.

Introduction

In his 1981 classic, *Plant Speciation*, Verne Grant (1981) devoted six chapters and 88 pages to the subject of interspecific hybridization. Comprising more than a fifth of the volume, this separately demarcated section, entitled *Natural hybridization and its products*, provided a synthesis of our understanding of the prevalence of hybridization in plants and the spectrum of possible evolutionary outcomes. This treatment, offered over 20 yr ago, testifies to a long-standing recognition among botanists of the high frequency and evolutionary significance of interspecific contact among plants. Of course, most of the evidence that Grant used and cited was morphological, although insights also emerged from cytogenetic and genetic investigations. For example, Grant noted that interspecific hybrids of *Opuntia spinosior* and *O. versicolor* (Grant & Grant, 1971) and *Aquilegia formosa* × *A. pubescens* (Grant, 1952) displayed a pattern of morphological intermediacy that was straightforward to interpret. Leafy-stemmed *Gilia* species, however, presented a more complex

pattern that posed a substantial challenge to traditional classification approaches (Grant, 1953). Elucidating the origin of the reticulating *Gilia* complex required a synthesis of information derived from morphometric (Grant, 1950; Grant, 1953), cytogenetic (Grant, 1953; Grant, 1965), and transmission genetic studies (Grant, 1966). These examples and others demonstrated that plant lineages may comprise reticulate networks rather than exclusively bifurcating independent entities.

The advent and application of molecular genetic tools over the last decades not only confirmed this feature of plant evolution, but also enormously expanded the list of plant taxa that appear to have experienced episodes of recent or historical introgression. Molecular investigations have been extraordinarily successful in confirming the hybrid nature of many species (Arnold, 1997; Rieseberg, 1997) and have permitted fine-scale characterization of the size and shape of contact zones (Rieseberg & Wendel, 1993; Arnold, 1997). An increasingly common observation in molecular phylogenetic studies is incongruence among trees derived from independent cytoplasmic (e.g. chloroplast [cp] DNA) and nuclear (e.g. ribosomal DNA) molecular markers. This pattern of incongruence may reflect numerous causes (Wendel & Doyle, 1998), but the biologically most noteworthy is 'cryptic' interspecific hybridization and introgression (Rieseberg, 1997; Wendel & Doyle, 1998; Raymond *et al.*, 2002), whereby molecular markers transferred due to interspecific gene flow generate contradictory phylogenetic inferences.

To date, only a fraction of plant genera have been subjected to molecular phylogenetic investigations that have employed multiple independent molecular markers that permit the detection of interspecific gene flow. Despite this shallow sampling of taxa and molecular markers, the number of cases of inferred historical hybridization events is remarkably high (Rieseberg & Soltis, 1991; Rieseberg, 1991; Wendel *et al.*, 1991; Wendel *et al.*, 1995b; Wendel & Doyle, 1998). Together these observations suggest that interspecific hybridization may be a more powerful and creative evolutionary process than envisioned by the early proponents of hybridization, such as Grant (1981), Anderson (1949) and Stebbins (1950). Moreover, one remarkable aspect of the many revelations from molecular-marker based studies is that inferred historical gene flow may be indicated between species that show strong geographic or reproductive barriers that would appear to prohibit the sexual exchange that is inferred to have taken place. These barriers include extreme geographic isolation as well as intrinsic barriers to mating and genetic exchange (Rieseberg & Soltis, 1991; Rieseberg & Wendel, 1993; Arnold, 1997; Rieseberg, 1997).

A case in point is *Gossypium*, a moderately sized genus with an aggregate global distribution that encompasses semiarid to arid regions of the tropics and subtropics. Because most cotton species exist in relatively small, scattered populations that appear to be highly inbred, and because species typically are geographically isolated from one another, *Gossypium* has not been a group for which a high frequency of hybridization has been suspected. Indeed, these hurdles have been used to argue that reticulate evolution among diploid cotton species is unlikely and unimportant (Fryxell, 1971; Fryxell, 1979). Nonetheless, molecular phylogenetic studies frequently arrive at the opposite conclusion, namely, that interspecific hybridization has been exceptionally widespread and important in the evolution of the genus. Moreover, it appears that some hybridization events involve species whose present suite of life-history features, biogeography, and intersterility pose such striking pre and post–reproductive isolation barriers that the likelihood of successful mergers would seem to be nil. Here, we summarize the evidence for these 'cryptic trysts' and 'genomic mergers' during *Gossypium* evolution, drawing attention to insights that have emerged from combined genetic, cytogenetic, biogeographic, and multiple molecular-marker based investigations. Our perspective is that since *Gossypium* exhibits unremarkable reproductive traits and dispersal biology, the evolutionary significance of interspecific hybridization is probably even greater than that envisioned not only by Grant (1953, 1981) but by contemporary authors (Arnold, 1997; Rieseberg, 1997) as well.

Evidence for cryptic hybridization in *Gossypium*

Gossypium includes about 50 species (Fryxell, 1992), including 18 species native to the New World, 12–14 species native to Africa/Asia, and 18 species native to Australia. Relationships within the cotton genus have been inferred using morphology (Fryxell, 1971; Fryxell, 1992), interfertility relationships and cytology (Endrizzi *et al.*, 1985) and molecular markers. Among the latter are a wealth of genetic data, including cpDNA restriction site variation (Wendel & Albert, 1992), DNA sequence variation from chloroplast genes (Seelanan *et al.*, 1997; Small *et al.*, 1998; Cronn *et al.*, 2002), DNA sequence variation from nuclear ribosomal DNA (5S gene and spacer, Cronn *et al.*, 1996, 5.8S gene and flanking internal transcribed spacers, Seelanan *et al.*, 1997), and DNA sequences from 10 low-copy nuclear genes (Small *et al.*, 1998; Seelanan *et al.*, 1999; Small & Wendel, 2000; Liu *et al.*, 2001; Cronn *et al.*, 2002; Cronn *et al.*, 2003). These studies have identified a common set of natural lineages that are congruent with geographic distributions (Fryxell, 1992) and cytogenetic 'genome' designations (abbreviated with the letters A–G and K; Endrizzi *et al.*, 1985), the latter of which are based on chromosome size and pairing behavior in interspecific hybrids. Our current hypothesis for phylogenetic interrelationships of the cotton genome groups is summarized in Fig. 1.

Three aspects of the *Gossypium* evolutionary and genetic history are relevant to the topic of cryptic interspecific hybridization. First, the eight diploid genome groups exist as four major lineages of species corresponding to three continents:

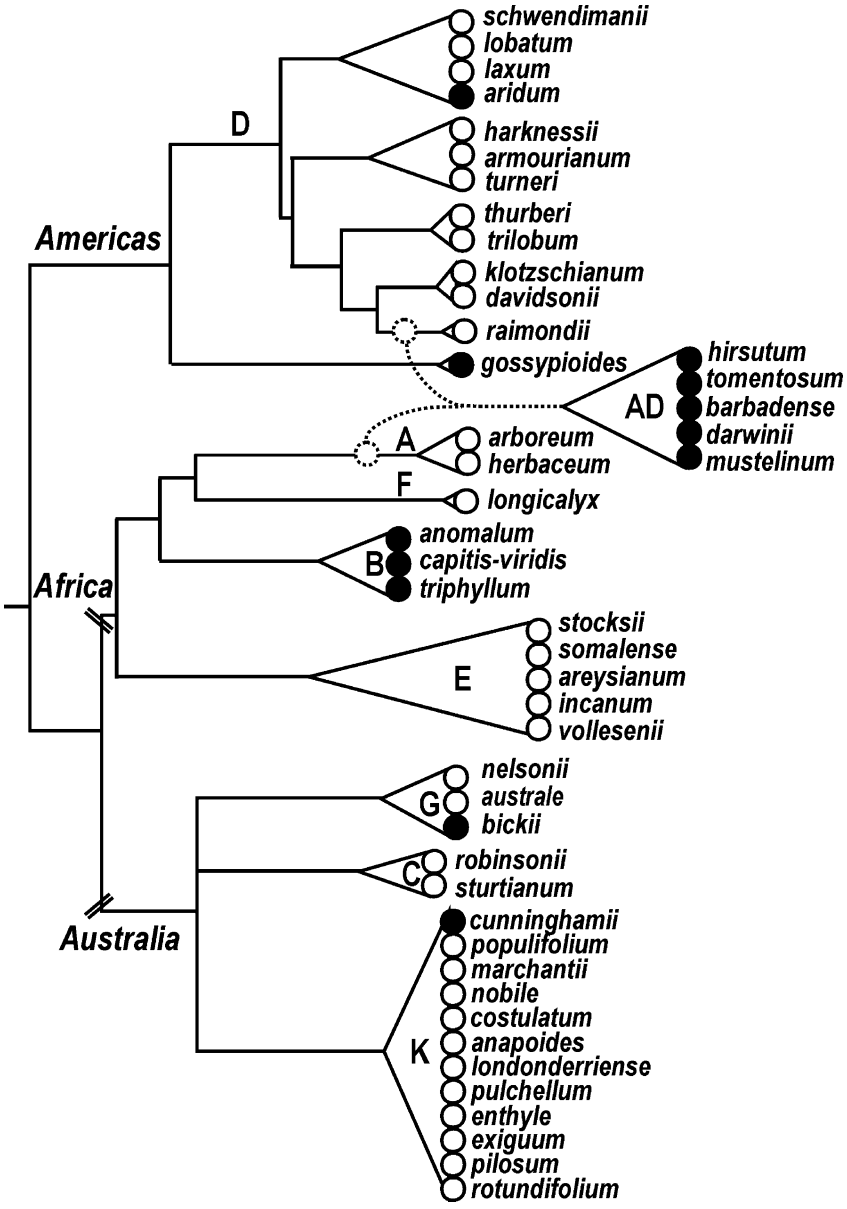

Fig. 1 Phylogenetic framework of *Gossypium* genome groups, as inferred from multiple low-copy nuclear genes. Genome designations for diploid lineages are identified by a single letter (A–G and K), and cotton species that show evidence of cryptic introgression are identified by a solid circle. Allotetraploid cottons are identified as the AD-genome, and the phylogenetic affinity of their A- and D-genome ancestors are shown with a dotted circle.

the Americas (D-genome), Australia (C-, G-, K-genomes), Africa/Arabia (A-, B-, and F-genomes) and the Arabian peninsula (E-genome). The earliest divergence in the genus separated the New World D-genome lineage from the ancestor of all Old World taxa, making New World and Old World diploids phylogenetic sister groups. Because of the high genetic divergence between these natural lineages, experimental interspecific crosses can only be accomplished between a limited number of closely related species; in all cases, F1 hybrids between genome groups are sterile (Endrizzi *et al.*, 1985). Second, molecular data from four chloroplast and 12 nuclear genes indicates that these lineages were established in relatively rapid succession following divergence from a common ancestor (Seelanan *et al.*, 1997; Cronn *et al.*, 2002). The evolutionary scenario envisioned is that there was a rapid radiation early in the history of the genus, with temporally closely spaced divergence events. Finally, the global distribution of the genus is believed to be caused by several recent (approx. 5–10 million years ago) long-distance oceanic dispersal events (Fryxell, 1979; Wendel & Albert, 1992). More recent examples of trans-oceanic dispersal events include the colonization of insular habitats such as Hawaiian Archipelago (tetraploid *G. tomentosum*), the Galapagos Islands (tetraploid *G. darwinii*, diploid *G. klotzschianum*) and the Cape Verde Islands (diploid *G. capitis-viridis*).

Distributed throughout the framework shown in Fig. 1 is a minimum of six unexpected and unlikely cases of reticulation among diploid species that collectively are implicated in the origin and/or subsequent evolution of 12 species, or about one-fourth of the genus. These cases of incongruence include:

five New World AD-genome allotetraploid species (Wendel *et al.*, 1995a; Small *et al.*, 1998); two New World D-genome diploid species, *G. gossypioides* (Wendel *et al.*, 1995b; Seelanan *et al.*, 1997; Cronn *et al.*, 2003) and *G. aridum* (Wendel & Albert, 1992, Àlvarez & Wendel, unpublished); two Australian diploid species, G-genome *Gossypium bickii* (Wendel *et al.*, 1991), and the K-genome species *G. cunninghamii* (Seelanan *et al.*, 1997; Seelanan *et al.*, 1999; Liu *et al.*, 2001); and three African B-genome diploid species, *G. anomalum*, *G. capitis-viridis* and *G. triphyllum* (Cronn *et al.*, 2002). These putative examples of cryptic genetic exchange underscore a remarkable feature of the genus, namely, potential for hybridization between lineages that have no obvious opportunity of sexual contact. It may be that one or more of these hybridization events stimulated the formation of new species, as is certainly the case for allopolyploid cottons. Each of these examples is discussed in turn below.

Intercontinental dispersal, allopolyploid speciation and the Gossypium tetraploids

The cottons of commerce – Upland cotton (*G. hirsutum*) and Pima cotton (*G. barbadense*) – represent two of five natural allotetraploid species that share a single origin, emerging from the chance union between two diploid species that evolved in isolation on separate continents. The worldwide importance of these species has motivated scores of researchers to attempt to decipher the mysteries surrounding their origin. A synopsis of this history is shown in Fig. 1, and can be found in Wendel & Cronn (2003).

Early cytogenetic surveys in *Gossypium* revealed the presence of two chromosome levels, $n = 13$ and $n = 26$ (Wendel & Cronn, 2003). Critically, it was noted that the formation of 13 bivalents in triploid hybrids between wild diploid and cultivated tetraploid species 'support the hypothesis that the species having 26 pairs are allotetraploids', and further suggested that the ancestral diploid donors involved 'wild American species … and Asiatic species' (Webber, 1935). The allopolyploid hypothesis gained support from experimental hybridizations between A-genome (African) and D-genome (American) diploids. Newly synthesized A × D F1 hybrids, while infertile, could be colchicine-doubled to produce self-fertile synthetic AD-genome tetraploids. Significantly, these amphiploids formed fertile hybrids with natural AD-genome tetraploids. In less than 20 yr, the chromosomal composition of cotton tetraploids was identified by morphometric and cytogenetic analysis. However, two significant questions remained unanswered by these approaches: Which diploid species best represent the progenitors; and when did the tetraploids form?

As most nuclear genes have been duplicated in tetraploid AD-genome cottons (Brubaker *et al.*, 1999), sequence analysis of duplicate loci (Small *et al.*, 1998; Cronn *et al.*, 1999; Small & Wendel, 2000a; Liu *et al.*, 2001; Cronn *et al.*, 2003) has yielded unprecedented insight into the parentage and timing of polyploid cotton formation. Low-copy gene sequences in AD-genome tetraploids clearly support the genomic origins of allopolyploid cotton and specifically point to A-genome African cottons (2 species) and the Peruvian D-genome diploid species *G. raimondii* as the closest living relatives of the ancestral genome donors (Fig. 1). Additionally, analysis of maternally inherited chloroplast DNA (Wendel, 1989) and mitochondrial DNA (Small & Wendel, 1999) identify the African parent as the maternal cytoplasm donor.

Using molecular clock arguments, these diploid progenitors show an estimated divergence time of 6–7 million years ago (Cronn *et al.*, 2002; Senchina *et al.*, 2003). At this time, North America and Africa had achieved their present locations, so the primary divergence between D- and A-genome diploid groups clearly involved trans-oceanic dispersal. Using similar logic, these diploid genomes were reunited in a common nucleus by polyploidization about 1.5 million years ago. Long-distance dispersal must have fostered the transient colonization of an A-genome cotton in the Americas, leading to hybridization with an indigenous *G. raimondii*-like diploid, and the subsequent extinction of the African emigrant. The nascent allopolyploid lineage radiated into three branches and five modern-day species.

Genomic chimerism and the multiply hybrid ancestry of *Gossypium gossypioides*

A striking example of interspecific sexual contact in *Gossypium* involves not only cytoplasmic introgression between species, but apparent recombination between diverged nuclear genomes (Wendel *et al.*, 1995b; Cronn *et al.*, 2003). The species in question is *G. gossypioides*, the sole member of subsection *Selera*, which is restricted to small, isolated populations in a single river drainage in Oaxaca, Mexico.

Until recently, *G. gossypioides* was considered unremarkable among American diploid cottons, as the inferred relationships between *G. gossypioides* and other D-genome species based on morphology, cytogenetic data, interfertility relationships, and allozyme analysis were congruent (Wendel *et al.*, 1995b). Wendel *et al.* (1995b), however, showed that the nuclear ribosomal DNA sequences from *G. gossypioides* are unlike those of other D-genome species. In fact, sequence analysis shows that *G. gossypioides* is strongly resolved as a member of the African lineage that includes A-, B-, and F-genome cottons (Seelanan *et al.*, 1997; Cronn *et al.*, 2003). Subsequent to this finding, *G. gossypioides* was shown to contain additional repetitive DNAs that are shared with African cotton species but are unknown among American (D-genome) and Australian (C- and G-genome) species (Zhao *et al.*, 1998). Complicating the story further, recent phylogenetic analyses based on nuclear 5S rDNA (Cronn *et al.*, 1996) and eight low-copy nuclear genes (Small & Wendel, 2000; Liu *et al.*,

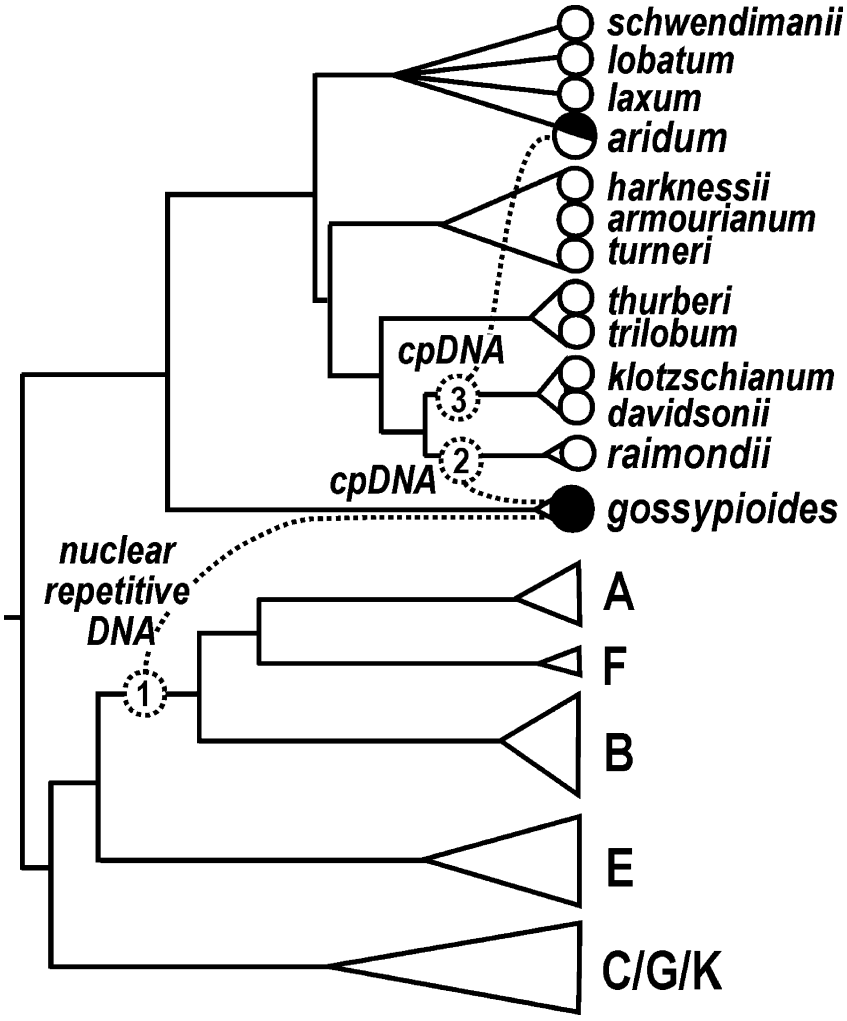

Fig. 2 Hybridization and introgression in New World cottons. Through two separate hybridization events, *Gossypium gossypioides* acquired nuclear repetitive DNA from an African source (progenitor 1), and a chloroplast genome that is highly similar to modern-day *G. raimondii* (progenitor 2). In addition, *G. aridum* from Colima, Mexico posseses a chloroplast genome that is divergent from all members of Subsection *Erioxylum*. Phylogenetic analysis shows that this chloroplast genome has a high affinity to *G. davidsonii* and *G. klotzschianum*, implicating a hybridization event between these divergent species.

2001; Cronn *et al.*, 2003) reveals that *G. gossypioides* occupies the basal position within the D-genome clade. This finding contrasts with expectations from morphology (Fryxell, 1971), interspecific hybridization studies (Brown & Menzel, 1952; Menzel & Brown, 1954), and evidence from the chloroplast genome (Wendel & Albert, 1992; Cronn *et al.*, 2003), all of which identify a close tie between *G. gossypioides* and *G. raimondii*, the latter of which occupies a terminal phylogenetic position within the D-genome assemblage (Small & Wendel, 2000; Cronn *et al.*, 2003).

These data implicate a complex history for *G. gossypioides*, one that includes two temporally separated introgression events involving genetically divergent cottons that are presently restricted to different hemispheres (Fig. 2). Based on repetitive nuclear DNA, it appears that *G. gossypioides* experienced nuclear introgression from an African species shortly after divergence from the American assemblage. This hybridization event either occurred at the diploid level or at the triploid level, possibly as a consequence of hybridization with the New World allopolyploid lineage (Wendel *et al.*, 1995b). Subsequent backcrossing of this hybrid to the *G. gossypioides*-like ancestor restored the single-copy component of the D-nuclear genome, but failed to purge all 'African' repetitive elements. More recently, hybridization with a Mexican species similar to modern-day *G. raimondii* (presently restricted to Peru) resulted in cpDNA introgression, and perhaps a second round of cryptic nuclear introgression (Cronn *et al.*, 2003).

Regardless of the details of this mysterious ancestry, *G. gossypioides* highlights the potential for genomic 'chimerism' within a diploid species for which no evidence of reticulation was detected before molecular investigation. Indeed, present geographic range distributions and genetic isolation mechanisms would suggest that the hybridization events implicated are implausible if not impossible. Yet the evidence for genomic contact is compelling and alternative explanations have not emerged. This example also illustrates the phylogenetic complexity that can result from multiple historical reticulation events, and thus serves as a sobering cautionary tale for inferences of history based exclusively on only one or two sources of molecular evidence.

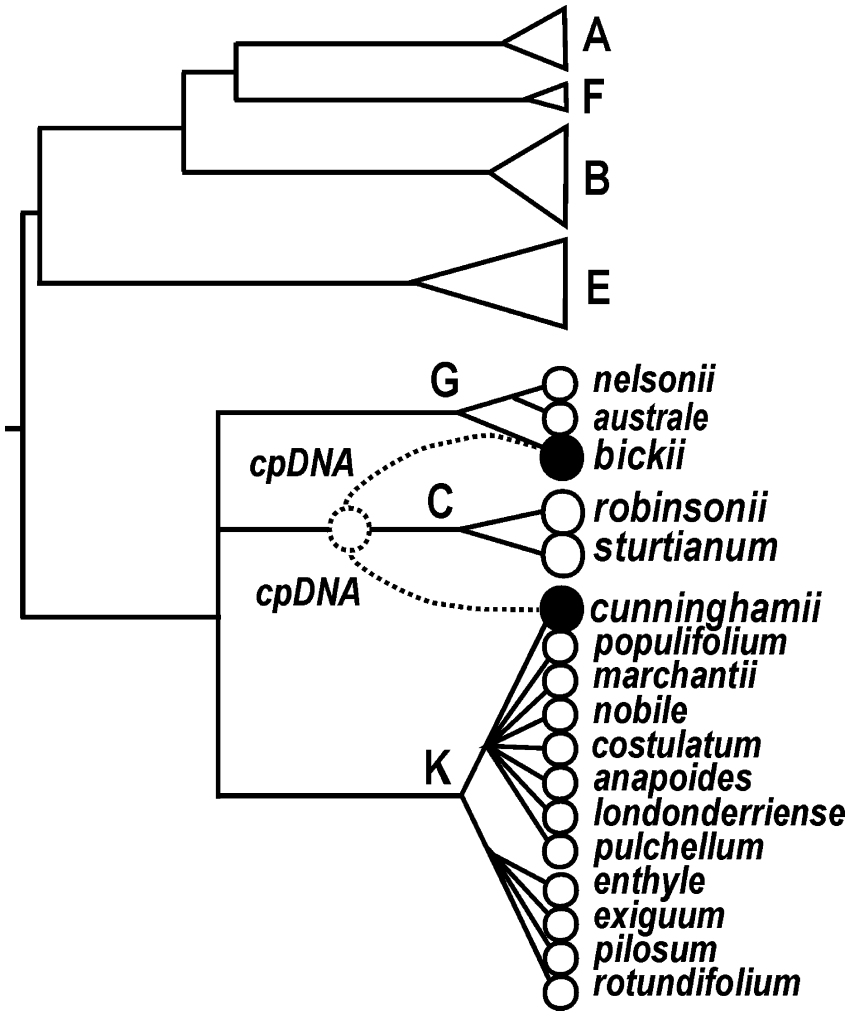

Fig. 3 Hybridization and introgression in Australian cottons. The G-genome species *Gossypium bickii* (from Section *Hibiscoidea*) and K-genome species *G. cunninghamii* (Section *Grandicalyx*) each possess a chloroplast genome that is uncharacteristic of their respective taxonomic Sections. Phylogenetic analysis shows these chloroplast sequences to be highly similar to modern-day *G. sturtianum*, implicating independent chloroplast transfer events between these divergent lineages.

Other cases of cryptic hybridization between allopatric *Gossypium* species

In addition to *G. gossypioides*, three additional diploid species in *Gossypium* are implicated to have experienced cytoplasmic transfer by virtue of incongruent cpDNA/nuclear data (Fig. 3). The first was discovered during a phylogenetic study of Australian species, where molecular markers from the plastid and nuclear genomes revealed an unusual evolutionary history for *G. bickii* (Wendel *et al.*, 1991; Seelanan *et al.*, 1999; Liu *et al.*, 2001). This species is one of three morphologically similar G-genome cottons, along with *G. australe* and *G. nelsonii* (Fryxell, 1971) included in section *Hibiscoidea*. By contrast to expectations based on this taxonomy, the chloroplast genome of *G. bickii* was shown to be nearly identical to the plastid genome of *G. sturtianum* and *G. robinsonii*, a pair of morphologically distant C-genome species from section *Sturtia*. By contrast, nuclear markers reveal the expected relationship, that is, that *G. bickii* shares a more recent common ancestor with its close morphological allies (*G. australe* and *G. nelsonii*) than it does with *G. sturtianum*/*G. robinsonii*. This discrepancy is explained by invoking a bi-phyletic ancestry for *G. bickii*, whereby a *G. sturtianum*-like species served as the maternal parent in an ancient hybridization with a paternal donor from the lineage leading to *G. australe* and *G. nelsonii*, which is shown in Fig. 3. To date, *G. sturtianum* and *G. robinsonii* alleles have yet to be detected in *G. bickii*, suggesting that the nuclear genomic contribution of the maternal parent was eliminated from the hybrid or its descendent lineage.

A similar example of 'cytoplasmic capture' has been implicated for the Australian K-genome cotton *G. cunninghamii*. This species has an unusual morphology and is geographically disjunct from all other K-genome species, in that it is restricted to the Cobourg Peninsula of Australia approx. 500 km distant from the Kimberley region where the remaining species are found. Analogous to *G. bickii*, the chloroplast genome of *G. cunninghamii* appears to have been donated by a *G. sturtianum*-like ancestor (Fig. 3), although in this case the hybridization event appears to have been more ancient (Wendel & Albert, 1992; Seelanan *et al.*, 1999; Liu *et al.*, 2001). As with *G. bickii*, nuclear ribosomal DNA (Wendel &

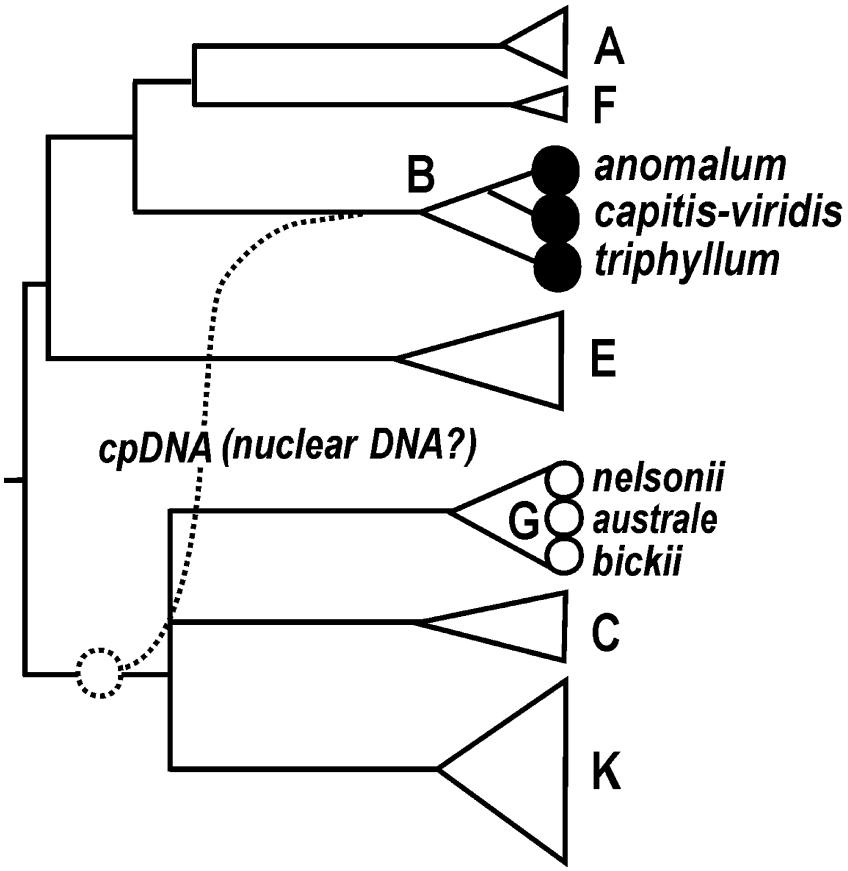

Fig. 4 Hybridization and introgression in African cottons. Nuclear markers identify the B-genome lineage as a member of a monophyletic African clade that includes A- and F-genome species. Nevertheless, phylogenetic analysis of B-genome chloroplast DNA shows this lineage to be the sister group of Australian C- and G-genome cottons, implicating a chloroplast transfer event between the progenitor of these Australian cottons and the progenitor of B-genome cottons. In addition to molecular markers, B-genome *G. triphyllum* is unusual in that it displays a number of morphological features restricted to G-genome species.

Albert, 1992; Seelanan *et al.*, 1999) and the low copy nuclear gene FAD2-1 (Liu *et al.*, 2001) show that *G. cunninghamii* shares a more recent common ancestor with its K-genome relatives than it does with *G. sturtianum* (Seelanan *et al.*, 1999; Liu *et al.*, 2001).

A final example of cryptic introgression at the species level is seen in *G. aridum*, one of four species of Mexican cottons that comprise subsection *Erioxylon*. These species are distinctive small trees, and their shared common ancestry is supported by nearly all studies to date (Fryxell, 1971; DeJoode, 1992; Wendel & Albert, 1992; Wendel *et al.*, 1995b; Cronn *et al.*, 1996; Small & Wendel, 2000). The sole exception to this unanimity is that *G. aridum* populations from the Mexican state of Colima have a chloroplast genome that is strikingly divergent from other populations of *G. aridum* and the other three species in subsection *Erioxylon* (DeJoode, 1992; Wendel & Albert, 1992). Curiously, this chloroplast genome shows high sequence similarity to the chloroplast genome of *G. davidsonii* and *G. klotzschianum*, two species from a different taxonomic section (*Integrifolia*) that are strongly differentiated at the morphological (Fryxell, 1971) and genetic level (Endrizzi *et al.*, 1985; Cronn *et al.*, 2003). The apparently 'alien' cytoplasm in Colima *G. aridum* is inferred to have arisen by introgressive hybridization with a member of the *Integrifolia* subsection, as shown in Fig. 2. As in the preceding examples, the extant species that best represent the putative cytoplasmic donor (*G. davidsonii* and *G. klotzschianum*) show geographic ranges (Baja California and the Galapagos Islands, respectively) that are distant from modern-day *G. aridum* and are reproductively isolated from *G. aridum* by intrinsic genetic barriers.

B-genome, African cottons – an additional ancient introgression event?

Three sub-Saharan cotton species comprise the B-genome diploid group, including *G. anomalum* (widespread in northern Africa), its sister species *G. capitis-viridis* (Cape Verde Islands), and the morphologically distinctive *G. triphyllum* (Angola, Botswana, and Namibia). These species form a genetically cohesive group within a larger, monophyletic lineage of African cottons that includes all species in the A-, B- and F-genome groups (Fig. 4). The association between the B-, A- and F-genome groups was long suspected, since they share a common geographic distribution, distinctively intermediate genome sizes (3.3–4.2 pg/2C), and similar chromosome pairing behavior in interspecific crosses (Endrizzi *et al.*, 1985). On the basis of these characteristics, Fryxell (1971, 1992) included *G. anomalum* and *G. capitis-viridis* as members of the subgenus

Gossypium, along with A- and F-genome cottons. However, because of the unusual and distinctive morphology of the sole remaining B-genome species, *G. triphyllum* was placed in an entirely different subgenus (Sturtia) that includes C-, G- and K-genome Australian cottons (Fryxell, 1979).

Comparative molecular analyses of African cottons based on four cpDNA gene sequences (Cronn *et al.*, 2002) and 12 nuclear markers (Seelanan *et al.*, 1997; Cronn *et al.*, 2002) mirror the morphological conflict of *G. triphyllum*, but this discrepancy extends to all three B-genome species. Nuclear markers clearly resolve the B-genome lineage as a member of the African clade that includes A- and F-genome cottons; in contrast, chloroplast DNA data robustly resolve the B-genome species as the sister lineage of Australian (C- and G-genome) species (Cronn *et al.*, 2002). Given the intercontinental allopatry of the relevant taxa, and cytogenetic evidence indicating poor meiotic pairing and complete sterility in (B × C)-genome hybrids, cytoplasmic introgression would seem an unlikely explanation for B-genome incongruence. Nevertheless, the distinctive morphology of *G. triphyllum* shows numerous similarities to Australian cottons (Fryxell, 1979), from its nearly linear epicalyx (similar to G-genome cottons), pink-purple corolla pigmentation (similar to C- and G-genome cottons), fruit pubescence (similar to G-genome *G. australe*), and trifoliolate leaf shape (similar to G-genome *G. bickii*). These characteristics are absent from other African cottons, and it is intriguing to consider that they may represent the vestigial remnants of an ancient hybridization between members of the B-genome African lineage and the Australian clade, as depicted in Fig. 4. If this seemingly improbable event occurred, the cpDNA introgression event would have had to occur before the diversification of modern-day B-genome species, as cpDNA restriction site analysis (Wendel & Albert, 1992) and limited *matK* sequencing (R. Cronn, unpublished) of B-genome *G. capitis-viridis* and *G. triphyllum* show that all B-genome species share the same plastome haplotype.

Prevalence of hybridization in evolution

Reflecting on the high degree of reticulation in groups such as *Gilia*, *Ceanothus*, *Aquilegia*, *Quercus*, agricultural crops and weeds, Grant (1953) suggested that 'the occurrence of sporadic hybridizations during the course of Angiosperm evolution may be the factor which has caused this group to grow up, not as a phylogenetic tree, but as a gigantic, snarled phylogenetic net'. This insightful comment seems even more appropriate today, and in the context of the present paper seems almost prophetic. As illustrated by the suite of examples from the *Gossypium*, and as supplemented by an ever-expanding list of examples from throughout the angiosperms (Arnold, 1997; Rieseberg, 1997; Raymond *et al.*, 2002), interspecific hybridization is far more prominent in plants than earlier suspected, even among species that might be judged as unlikely to engage in interspecific sexual contact. Thus, modern–day reproductive isolation barriers that are seemingly insurmountable must either have arisen subsequent to the inferred reticulations, or they were somehow surmounted by mechanisms that we do not as yet appreciate.

In his discussion on the importance of hybridization in *Gilia* evolution, Grant cautioned that 'even the most intensive study of a [hybrid] complex can permit only a minimal estimate of the importance of hybridization in the evolution of such a group' (Grant, 1953). This perspicacious comment reflects a recognition that putative parents may themselves be of hybrid origin, while other cases may remain cryptic due to extinction of progenitor lineages, subsequent evolutionary divergence, or insufficient evidence. For this reason, inferences concerning the frequency and importance of hybridization are always underestimated, irrespective of the approach used.

Notwithstanding these limitations, the identification of parental species and their putative hybrid derivatives clearly is a challenging but critical step in understanding the role of hybridization and introgression in plant evolution. As noted by Grant (1953), the historical reliance upon morphology and cytological methods has allowed us to only 'recognize the more recent and actively evolving homogamic (reticulating) complexes which have not suffered widespread extinction.' Indeed, many of the best characterized examples of hybrids and hybridization involve taxa that show a relatively high degree of interfertility and remain more or less sympatric, or for which one or both parents co-occur with the putative hybrid (Arnold, 1997; Rieseberg, 1997). More ancient hybridization events become increasingly obscure with time and subsequent evolutionary change, such that morphology and/or cytogenetic tools alone are ever more unlikely to yield clues of past reticulation events. It is in this realm that molecular phylogenetic approaches prove especially useful, as they may provide insight into ancient and otherwise cryptic introgression. As molecular tools become increasingly applied in phylogenetic studies, and especially once multiple nuclear genes are employed in addition to cpDNA markers (Small *et al.*, 2004), a clearer picture will emerge of the scope and prevalence of hybridization in plant evolution.

Perspectives

As the extent of hybridization in plant evolution becomes clearer, it is likely that additional attention will be focused on a suite of interrelated questions, many of which are outlined in this volume. These questions collectively comprise a challenging but integrated research agenda for the next century, and necessitate a multidisciplinary approach to fundamental questions in evolutionary biology. One fruitful avenue will explore the reproductive biology of hybrid formation as well as subsequent events in hybrid stabilization and/or introgression. In addition to exploring the floral

biology and ecological context of interspecific gene exchange, research is needed into the mechanisms by which intrinsic barriers to sexual contact, sometimes seemingly insurmountable, are overcome. Similarly, as more examples of ancient and modern reticulation accumulate, the spectrum of genomic consequences of gene exchange requires detailed elaboration, as does the functional significance of genetic transfer. In this latter arena, genomic technologies are likely to yield rich and lasting insights. Once accomplished, answers may emerge to the central question of why hybridization has had such a profound impact on biodiversity.

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